

Shin WATANABE*: **The genus *Chlorella* (Chlorococcales)
from Japanese soils (1)**

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Introduction

As has been reviewed by Fott and Nováková, isolations and cultures of species of the genus *Chlorella* are generally so easy that they are one of the most convenient materials for physiological investigations. Thus it happened that numerous taxa have been described randomly by not only taxonomists but also workers who occupy themselves mainly with bacteriology or plant physiology. In the taxonomy of *Chlorella* at the specific rank there has been an attempt to classify the organisms according to similarities or differences in the physiological characters rather than those in the morphological ones. This proceeded from the presumption that the specific separation cannot be based on the morphology only, because the morphological differences were recognized as too delicate to classify taxa. Recently an excellent monograph of *Chlorella* based on morphological and structural characters has been made by Fott and Nováková (1969). According to their opinion, biochemical and physiological investigations of *Chlorella* indicate that, in the majority of cases, one quality does not characterize a morphologically-defined species but may occur in a number of species: On the other hand, various morphologically-defined species can include infraspecific units (varieties, forms) exhibiting the same physiological and biochemical properties.

Very little is studied, although many physiological works exist, concerning the taxonomy of the genus *Chlorella* in Japan. Since 1972, I have been engaged in the study of this algal group from the view-point of taxonomy. In total, 34 specimens have been isolated from soils of different localities in Japan. Identification of the isolates were mainly made in the light of the monograph by Fott and Nováková (1969), and as a result, it

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became clear that 22 isolates coincided with 9 taxa recorded by Fott and Nováková, 7 isolates coincided with *Pseudochlorella subsphaerica* Reising (1964) and 5 isolates were identified to be hitherto non-described species. In the present paper, which constitutes the first of a short series, are described and illustrated 12 taxa of *Chlorella* in Japan.

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Materials and methods

Soil samples were collected from the following localities in Japan:

Symbols of the localities

HKN: Near the factory of lime at Hikone in Shiga Prefecture.

IWA: The secondary forest of *Pinus densiflora* in the northern part of Kyoto City.

KIY: The primary forest dominantly occupied by *Castanopsis cuspidata* in the eastern part of Kyoto City.

KKY: The Yodo Racecourse in the southern part of Kyoto Prefecture.

KUC: The campus of Kyoto University, Kyoto City.

OOD: The primary forest dominantly occupied by *Pseudotsuga japonica* in the southern part of Nara Prefecture.

SAI: Plowed fields in Saitama Prefecture. Soils are characterized by the Kanto Loam.

SAK: The Taisho Lava in Mt. Sakurajima, an active volcano in Kagoshima Prefecture. Soils contain the volcanic ash.

TOT: The coastal sand dune in Tottori Prefecture. Soils of the bare dune and of the place far away from the sea shore in the garden of the Sand Dune Research Institute of Tottori University, where *Pinus thunbergii* is planted.

YAW: The bank of the Yodo River in Yawata Street, Kyoto Prefecture, dominantly occupied by *Solidago altissima*. Wet sandy soil.

Soils in YAW, TOT, KIY and IWA were collected several times in different seasons, but those in other localities were collected once. Soils were obtained from the surface of ground in the grassy and bare land, and from the lower part of litter in the forest soil.

In 10 ml of the distilled water, 2g of the soil sample were suspended and diluted 10-, or 100-fold with it. One ml of each suspensions was scattered with a pipette on the agar plate. Cultures were maintained in the Bold's Basal Medium (replacing double distilled water by tap water), at 18-25°C under a 12:12 h light-dark cycle illuminated by daylight fluorescent lamps at 2000-3000 lux. Algae on the agar plate were transferred to the test tubes containing solid medium and sometimes liquid one, as needed. Isolates were observed during prolonged periods from few days to several months after inoculation in the fresh medium. The observation was made with a light microscope equipped with 10×, 40× and 100× (oil-immersion) objectives and 15× oculars. The drawings were made directly from living materials.

Chlorella Beijerinck 1890

In the agreement with Fott and Nováková (1969), the genus *Chlorella* is circumscribed as follows in the present study:

Cells spherical, subspherical or ellipsoidal, covered with a smooth cell wall without mucilage, single or in small irregular groups. Chloroplast single, parietal, covering nearly the whole periphery of the cell or only part of it. Pyrenoid present or absent. Nucleus single in young cells; older cells can form multinucleate sporangia.

Reproduction by autospores, zoospores lacking. Autospores liberated by rupture or by dissolution of the mother-cell wall.

The main characters for classifying *Chlorella* at the rank of species and variety are also in agreement with Fott and Nováková. These are as follows; form of cells, fate of the mother-cell wall, chloroplast morphology, presence or absence of pyrenoid, number and size of autospores, mode of autospore release and dimension of the cells.

Key to species and varieties of *Chlorella* collected in Japan

1. Pyrenoid present..... 2

2. Young vegetative cells broadly ellipsoidal, subspherical, or spherical 3
3. Adult vegetative cells spherical or nearly spherical 4
4. Chloroplast in one piece and undivided 5
5. Chloroplast cup-shaped or girdle-shaped *C. vulgaris*
5. Chloroplast mantle-shaped *C. kessleri*
4. Chloroplast apparently occupying entire portion of cell's periphery with several incisions at margin 6
6. Vacuoles rare or lacking *C. fusca* var. *fusca*
6. Vacuoles numerous, always present *C. fusca* var. *vacuolata*
3. Adult vegetative cells ellipsoidal 7
7. Chloroplast small, with smooth margin; pyrenoid naked; autospores equal in size *C. saccharophila* var. *saccharophila*
7. Chloroplast large, with irregular margin; pyrenoid with starch; autospores unequal in size *C. saccharophila* var. *ellipsoidea*
2. Young vegetative cells sometimes elongate-reniform, or elongate-ellipsoidal *C. reisigii*
1. Pyrenoid absent 8
8. Young vegetative cells broadly ellipsoidal, subspherical, or spherical.. 9
9. Chloroplast occupying almost entire portion of cell's periphery, with deep, irregular clefts *C. zofingiensis*
9. Chloroplast with smooth margin 10
10. Chloroplast broadly band-shaped or cup-shaped, occupying up to one-half of cell's periphery *C. protothecoides*
10. Chloroplast cup-shaped, girdle-shaped, or mantle-shaped, occupying 2/3-4/5 of cell's periphery *C. minutissima*
8. Young vegetative cells sometimes elongate-reniform or elongate-ellipsoidal *C. reniformis*

Chlorella vulgaris Beijerinck var. **vulgaris**; Beijerinck 1890, p. 758, fig. 7, 2; Fott et Nováková 1969, p. 21, pl. 1. a-m. (Fig. I, 1-7)

Adult cells spherical; young cells and autospores ellipsoidal to subspherical. Adult cells 8.0 μm in diam.; young cells and autospores 1.5 $\times$ 2.5 μm in size or 3.0 μm in diam. Cell wall thin throughout culture. Chloroplast cup-shaped, girdle-shaped, band-shaped, or mantle-shaped. Pyrenoid one or

rarely two, spherical or rather ellipsoidal, covered with apparently continuous or two hemispherical starch grains. Nucleus invisible without stain. Vacuoles and oil droplets sometimes present.

Reproduction by 2-4 autospores. Autospores ellipsoidal, subspherical, equal in size in a sporangium. Mother-cell wall breaks down into 2 or 4 fragments after liberation of autospores.

Plant mass smooth, green in young cultures, sometimes yellow-green in old cultures.

Culture No. SAK-31, KKY-5.

Chlorella vulgaris Beijerinck var. *vulgaris* f. **viridis** (Chodat) Fott et Nováková 1969, pl. 2, a-h. (Fig. I, 8-20)

Adult cells spherical; young cells and autospores ellipsoidal to subspherical. Adult cells $7.0\text{ }\mu\text{m}$ in diam., rarely large cells up to $14\text{ }\mu\text{m}$ in diam.; young cells and autospores $2.5 \times 3.0\text{ }\mu\text{m}$ in size. Cell wall thin throughout culture. Chloroplast broadly band-shaped, cup-shaped, girdle-shaped, frequently shallowly incised at 2-4 points. Chloroplast of large cells appressed to the cell wall by a vacuole, sometimes divided into several portions. Pyrenoid of normal cells spherical, one sometimes two, covered with two hemispherical starch grains. Pyrenoid of large cells one to several, poorly surrounded with starch. Nucleus and nucleolus rarely visible without stain. Vacuoles observed only in large cells in young culture, and present in all individuals in old culture. Oil droplets present.

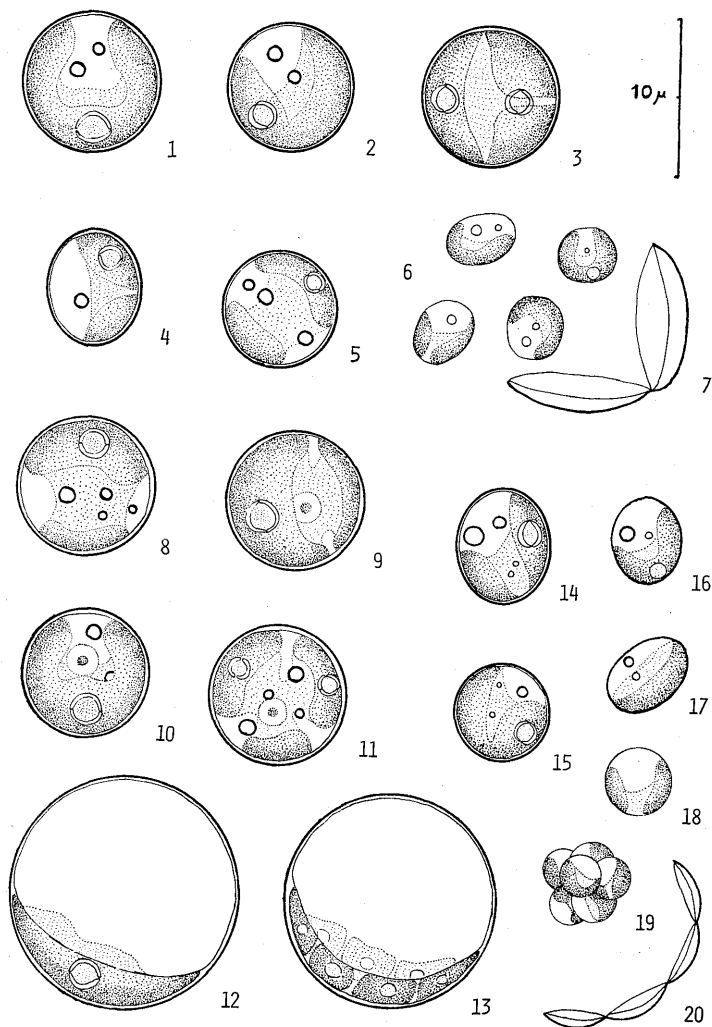
Reproduction by 2-8 autospores. Autospores usually equal in size in a sporangium. Mother-cell wall breaking down into 2-4 fragments after liberation of autospores.

Plant mass smooth, green, sometimes turning to yellow-green in old cultures.

Culture No. TOT-4.

C. vulgaris var. *vulgaris* f. *viridis* is distinguished from f. *vulgaris* by possessing large cells which are twice as large as normal ones, such as $14\text{ }\mu\text{m}$ in diam. In these large cells a large vacuole appresses the chloroplast to the cell wall, and the chloroplast was sometimes observed to be divided into several portions in each of which a pyrenoid is embedded. Although no individuals intermediate between normal and large cells were present in actively growing culture, sizes of cells in old cultures (about 5 months)

continuously changed from $3\mu\text{m}$ to $12\mu\text{m}$ in diam. One of characters that separates var. *autotrophica* from var. *vulgaris* is the visibility of the nucleus and nucleolus without stain: The former has a visible nucleus and nucleolus, and those of the latter are invisible. In TOT-4 nucleus and nucleolus were observed on a rare occasion in the aperture of the chloroplast. This may



lead the identification of the isolate to the var. *autotrophica*, but it is identified to var. *vulgaris* f. *viridis* because of the presence of large cells. Although mother-cell wall disintegrated usually into 4 fragments, they had a tendency not to separate absolutely from each other and to hold the autospores together. Autospores liberated from the mother-cell walls were sometimes seen to be aggregated for a while.

Chlorella kessleri Fott et Nováková 1969, p. 26, pl. 4. (Fig. II, 1-7)

Adult cells spherical; young cells ellipsoidal, rhombic, or trigonal with rounded ends. Adult cells up to $8.0\ \mu\text{m}$ in diam.; young cells, $3.0\ \mu\text{m}$ in diam. in average. Cell wall thin throughout culture. Chloroplast mantle-shaped, with one or two small apertures or with deep clefts occupying almost entire portion of cell's periphery. Pyrenoids one or rarely two, elongate-ellipsoidal, covered with two hemispherical starch grains, deposited in the basal part of the plastid. Nucleus and nucleolus sometimes distinctly visible. A few vacuoles present. Oil droplets always present.

Reproduction by autospores. Mother-cell wall bursting crosswise incompletely, forming a saucer-like arrangement.

Plant mass smooth, green, not changing in color in old cultures.

Culture No. KKY-15.

Chlorella fusca Shihira et Krauss var. *fusca*; Shihira et Krauss 1963, p. 35, fig. 43, 44; Fott et Nováková 1969, p. 28, pl. 5. (Fig. II, 8-18)

Adult cells ellipsoidal, sometimes subspherical to spherical in old culture; young cells and autospores ellipsoidal. Ellipsoidal cells $2.0-6.0 \times 3.0-8.0\ \mu\text{m}$ in size, rarely $10 \times 12\ \mu\text{m}$ in size; spherical cells up to $10\ \mu\text{m}$ in diam. Cell

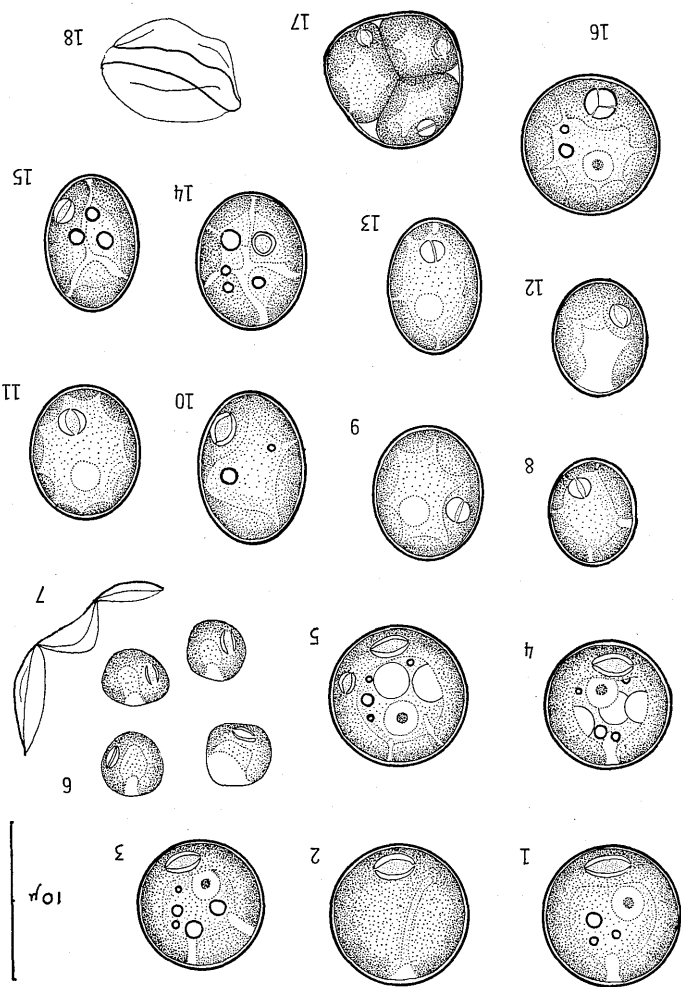
Fig. I. 1-7: *Chlorella vulgaris* Beijerinck var. *vulgaris*.

1, spherical cell with a cup-shaped chloroplast. 2, spherical cell with a girdle-shaped chloroplast. 3, spherical cell with a chloroplast which is deeply cloven at three points, and which possesses 2 pyrenoids. 4, young ellipsoidal cell with a band-shaped chloroplast. 5, young spherical cell with a broadly band-shaped chloroplast. 6, just liberated autospores. 7, empty mother-cell wall.

8-20: *Chlorella vulgaris* Beijerinck var. *vulgaris* f. *viridis* (Chodat) Fott et Nováková.

8, spherical cell with a broadly band-shaped chloroplast. 9, spherical cell with a mantle-shaped chloroplast incised at 2 points; nucleus and nucleolus visible. 10, spherical cell with a cup-shaped chloroplast; nucleus and nucleolus visible without stain. 11, spherical cell with a mantle-shaped chloroplast which is cloven at three points, and which possesses 2 pyrenoids; nucleus and nucleolus visible. 12, large cell with a saucer-shaped chloroplast appressed to the cell wall by a large vacuole. 13, large cell with segmented chloroplasts which have a pyrenoid. 14-16, young cells with an observable pyrenoid. 17 and 18, young cells without visible pyrenoid. 19, aggregation of just liberated autospores. 20, empty mother-cell wall left in 2-4 fragments;

wall thin throughout culture. Chloroplast parietal with many incisions at the margin, in old culture sometimes appearing to be segmented by deep clefts. Pyrenoid single, spherical, deposited in the thickening of the plastid, covered with two hemispherical starch grains which generally appear continuous. Nucleus and nucleolus rarely visible without stain. Vacuoles absent. Oil droplets present.



Reproduction by 2-8 autospores. Autospores equal in size in a sporangium. Fragment of mother-cell wall pouch-like.

Plant mass smooth, green, turning to reddish brown in old cultures.

Culture No. OOD-12, SAK-5, TOT-58, YAW-57, YAW-101.

When the margin of the chloroplast in OOD-12 was very deeply cloven, it appeared as if the plastid were segmented into several discoid portions and as if the pyrenoid were floating in the cell lumen rather than deposited in the chloroplast. This state of the chloroplast and the pyrenoid bears a striking resemblance to that of *Muriellopsis pyrenigera* Reisigl (1964). The genus *Muriellopsis* is distinguished by the presence of the pyrenoid from the genus *Muriella* (Petersen 1932) which has several discoid chloroplasts. Reisigl described the pyrenoid of *M. pyrenigera* as embedded in the chloroplast or outside of it. This curious feature of pyrenoid may arouse the following considerations: The pyrenoid is covered with the very thin layer of the chloroplast which might be overlooked by light microscopy, or *M. pyrenigera* was a species described by Reisigl who might observed *C. fusca* var. *fusca* whose chloroplast was so deeply cloven that it appeared to be segmented into discoid portions.

I have another question regarding whether *Pseudochlorococcum polymorphum* Archibald (1970) coincides with *Chlorella fusca* var. *fusca*. The comparison of my living isolates which were identified to var. *fusca* according to the illustrations drawn by Fott and Nováková, with *P. polymorphum*, which was sent from Indiana University's Culture Collection, did not provide efficient differences to separate the taxa. Although in *P. polymorphum* occur some cells whose shape varies strikingly, most individuals are similar to characteristics of var. *fusca* in shape and size of cells and

Fig. II. 1-7 : *Chlorella kessleri* Fott et Nováková.

1, spherical cell with a mantle-shaped chloroplast, with a small opening; nucleus and nucleolus visible. 2, surface view of cell; chloroplast with a deep cleft. 3, spherical cell with a mantle-shaped chloroplast, and with 2 shallow incisions. 4, spherical cell with a few vacuoles. 5, spherical cell with two pyrenoids. 6, just liberated autospores with rather angular shape. 7, empty mother-cell wall.

8-18 : *Chlorella fusca* Shihira et Krauss var. *fusca*.

8, 10 and 12, ellipsoidal cell with a parietal chloroplast. 9 and 11, ellipsoidal cell with an obscurely observable nucleus. 13, ellipsoidal cell with a pyrenoid which is covered with 2 thickened starch grains. 14 and 15, cells in old culture with segmented chloroplasts. 16, spherical cell in old culture with a pyrenoid which is covered with 3 thickened starch grains; nucleus and nucleolus visible. 17, autospores in a sporangium. 18, empty mother-cell wall.

in the morphology of the chloroplast with a pyrenoid. Unfortunately I could not examine the living strain of var. *fusca* identified by the original author; however, variations in shape of cells of *P. polymorphum* seem to be included in those of var. *fusca*. (To be continued)

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日本各地の土壌からクロレラ属の藻株 34 を分離し、主として Fott & Nováková (1969) の分類系に基づいてそれらを同定した結果、22 の藻株は既知のクロレラ属の 9 種 3 変種のうちの 7 種 2 変種のいずれかであることがわかった。本報告ではこれらの中の *Chlorella vulgaris*, *C. kessleri* 及び *C. fusca* var. *fusca* の 3 種が記述された。残余の種類及び 7 藻株から得られた *Pseudochlorella* の 1 種と 5 藻株から得られた *Chlorella* の未記載種については後報で記述する。

○シロバナノハラクサフジ (大橋広好・立石庸一) Hiroyoshi OHASHI & Yo-ichi TATEISHI: A white-flowered form of *Vicia amurensis* (Leguminosae)

ノハラクサフジは普通青紫色の花をつける。原記載は確かめられなかったが、Fl. USSR 13: 446 (1948) にはこの花が白いという記載はなく、またシベリアで得られたタイプ標本も不明であるという。白花品は日本では非常に珍しいものであり、かつソ連ではその記録がなさそうなので、シベリアでも恐らく稀なものであろう。従ってこれを品種として新たに記録しておくこととしたい。

シロバナノハラクサフジ

Vicia amurensis Oettingen f. **alba** Ohashi et Tateishi, f. nov.

Flores albi.

Type: Japan. Nagano-ken, Karuizawa. 10 Aug. 1974 Y. Tateishi 1890 (Ti).

□Y. Horikawa: **Atlas of the Japanese flora**, II. pp. 501-862, with maps. Gakken Co., Ltd, Tokyo, ¥35,000. 故堀川芳雄氏の力作、日本植物分布図の第 2 巻が出版された。内容の形式は 1 巻と全く同じである。ナギからはじまってクロカミランに終る 361 種の植物の分布が三方向、即ち水平、東西、南北に納められている。終りに和洋の索引が I, II 巻を通じてのせてある。みていると中々興味がある。たとえばゴメウツギ、ヒカゲツツジ、ヤブレガサが裏日本に見られぬことに気がつくし、トサボウフウが四国で低山から山頂近くまで分布すること、ナツアサドリやハマギクの分布の異常(?)さに目をみはる。堀川氏はこの本の校正中に病でなくなれたが、その努力は大変なものであったに違いない。このような資料を我々の使用にまかせて下さったことに感謝の念を禁ずることができない。改めて御冥福を祈る。(前川文夫)